PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	SEVERITY OF RESPIRATORY FAILURE AT ADMISSION AND IN-HOSPITAL MORTALITY IN PATIENTS WITH COVID-19: A PROSPECTIVE OBSERVATIONAL MULTICENTRE STUDY
AUTHORS	Santus, Pierachille; Radovanovic, Dejan; Saderi, Laura; Marino, Pietro; Cogliati, Chiara; De Filippis, Giuseppe; Rizzi, Maurizio; Franceschi, Elisa; Pini, Stefano; Giuliani, Fabio; Del Medico, Marta; Nucera, Gabriella; Valenti, Vincenzo; Tursi, Francesco; Sotgiu, Giovanni

VERSION 1 – REVIEW

REVIEWER	Celal Satıcı Istanbul, University of Health Science, Gaziosmanpasa Research and Training Hospital, Turkey
REVIEW RETURNED	21-Aug-2020

GENERAL COMMENTS	The reviewer provided a marked copy with additional comments. Please contact the publisher for full details.

REVIEWER	Davide Colombi Guglielmo da Saliceto Hospital Italy
REVIEW RETURNED	27-Aug-2020

GENERAL COMMENTS	Dear Authors, this study reported lower PaO2/FiO2 ratio as a predictor of in-hospital mortality in COVID-19 patients. This is a very interesting result, considering that risk stratification in Emergency settings is matter of concern for appropriate COVID-19 patients management. Another strength of the study is the prospective design. However several issues listed below should be addressed. Methods How did you make the diagnosis of COVID-19? By using nasal-pharyngeal swabs? To exclude COVID-19 did you use single or double negative swabs? In addition, in which period were patients enrolled? Results I suggest to perform Cox analysis by using categorical variables dervied from continuos variables (e.g Age > 65 years old) and add Kaplan-Meier for variables signicantly associated with death. In addition you should run ROC curve to evaluate the performance of the model in predicting death.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

(comments on PDF file)

We thank the Reviewer for the fruitful and useful suggestions. The following points were extrapolated from all the comments contained in the revised manuscript in PDF format. The Reviewer can consider all the changes regarding deletions and insertions throughout the text as accepted, unless otherwise reported below.

1. Abstract, Objectives: It should have been 'mainly based on direct measurement of hypoxia'.

Answer: we thank the Reviewer for the observation. In this line we wanted to underline that so far, in patients with COVID-19 pneumonia, many observational studies and triage recommendations based the grading of disease severity and respiratory failure only on an indirect assessment of hypoxia (i.e. peripheral oxygen saturation - SpO2). As the sentence may be confusing, we decided to remove it from the paragraph.

2. Abstract, line 14: Method section should be shortened with using only main points

Answer: we thank the Reviewer for the suggestion. We tried to shorten up as much as possible the sections of the Abstract containing materials and methods.

3. Abstract, primary and secondary outcome measures: CPAP or NIMV ?

Answer: the secondary outcome was designed to assess the rate of CPAP application (in Italy we used helmets, as described in detail in the Methods of the manuscript). During the COVID pandemic, NIV was used in a negligible portion of patients undergoing non-invasive ventilatory support to treat hARF in our facility.

4. Abstract, results: p<0.001

Answer: corrected as suggested

5. Abstract, results: The decimal part should be lengthened to specify the interval

Answer: We thank the Author for the suggestion. The novel results have been described as suggested.

6. Abstract results' comments

Answer: The results of the abstract and the manuscript have been changed according to the new analysis performed considering categorical rather than continuous variables for age and PaO/FiO2, as suggested by Reviewer #2. Please see figures and results section of the manuscript for further details.

7. Abstract, conclusions: Conclusion part should be specified as 'Lower P/F is independently... '

Answer: The conclusions have been modified according to the new results as it follows:

"A moderate to severe impairment in PaO2/FiO2 was independently associated with a threefold increase in risk of in-hospital mortality. Clinical severity of COVID-19 should be re-considered based on hARF severity."

8. STRENGTHS AND LIMITATIONS: Severe patients were %15 of total patients (not so high regarding COVID-19 patients). Also there may not be a selection bias if the patients were

consecutively enrolled. In fact, if the PEEP level was taken into account while classifying, the rate of seriously ill patients could be even less.

Answer: We thank the Reviewer for the comment and for giving us the chance to explain our statement. The median PaO2/FiO2 at admittance in our study was 262 (140-343) mmHg, with 37.4% of patients having a moderate to severe respiratory failure. Severity of patients with COVID-19, following the Chinese CDC/WHO definition, severe patients may have a SpO2<93% or a PaO2/FiO2<300 mmHg. This definition may imply that 61.8% (a 24.4% in addition to the previous figure if we sum also patients with PaO2/FiO2 200-300 mHg) of our patients could be defined as severe. Putting our data into perspective, the proportion of severe patients is much higher than in many reports involving patients outside the ICU. We believe that this was due to the fact that the intermediate care respiratory units were tertiary referral centers, with a high concentration of severe and complicated cases if compared with other centers in Italy. Considered the above discussion, we thought that the limitation would be consistent with our findings and kept it in its actual form.

9. STRENGTHS AND LIMITATIONS, fourth bullet point: This limitation is really problematic that the main component of this study (P/F) may not be accurate.

Answer: We thank the Reviewer for the comment. We do not entirely agree with the Reviewer's point of view on P/F accuracy. We stated that "Not all patients were evaluated in room air conditions at admittance, thus potentially underestimating the severity of the study sample". These are the results of an observational clinical study, in which patients were sometimes referred to our emergency department (and consequently to our wards) in critical conditions, i.e. already undergoing CPAP treatment or having Venturi or Reservoir masks already applied. Patients' conditions at admission are stated in Table 1 of the manuscript. To undertake a blood gas analysis in room air for all patients was not possible for a practical and ethical point of view, and thus a portion of patients were evaluated during oxygen supplementation. This is why we decided to use the PAO/FiO2 ratio and not raw PaO2 or SpO2 for our study calculations. As we state in the limitation section, the fact that some patients were not evaluated in room air conditions may have caused an underestimation of the patients' severity, thus enforcing our findings and the association between respiratory failure and mortality. Considered the reason explained above, we decided not to modify the sentence in the "methods limitation" section.

10. Introduction: It's a bit assertive to say there was no information on this subject. For example, the article: 'Grasselli G, Greco M, Zanella A, et al. Risk Factors Associated With Mortality Among Patients With COVID-19 in Intensive Care Units in Lombardy, Italy [published online ahead of print, 2020 Jul 15]. JAMA Intern Med. 2020;e203539. doi:10.1001/jamainternmed.2020.3539.' is an example of this subject.

Answer: we thank the Reviewer for the comment, and we agree the sentence might be too assertive for the topic covered. We changed it accordingly as follows: "Data on the association between severity of respiratory failure at admission and patients' outcomes are still limited".

11. Methods, patients: But, Table 1 states that all patients are seropositive.

Answer: we agree with the Reviewer. We stated that we enrolled "Adult hospitalized patients with a virologically-confirmed diagnosis of SARS-CoV-2 infection or with COVID-19-related symptoms and radiological signs" because it was part of the methods of the registered protocol. However, due to evolving local standard operating procedures, all patients admitted in the participating centers had a positive NP swab for SARS-CoV-2. We therefore changed the sentence as follows: "Adult hospitalized patients with a virologically-confirmed diagnosis of SARS-CoV-2 infection were considered eligible for study enrolment."

12. Methods, Patients: In the abstract it is said 'There is no specific exclusion criteria'. I wonder how the author found out about the outcomes of patients who were discharged to another facility?

Answer: we thank the Reviewer for the observation. We changed the abstract section of interest as follows: “Patients with <18 years old or unable to provide informed consent were excluded from the study”.

Patients discharged to other facilities were all patients needing a reduction in intensity of care, and were all transferred in stable clinical conditions to undergo physiotherapy or to expect the NF swab to become negative before returning to their homes. None of the discharged patients was dead at the moment of writing the report.

13. Methods, procedures: Is the FiO2 level applied during arterial blood gas analysis known for all patients?

Answer: The applied FiO2 for each patient was recorded at admission to calculate the PaO2/FiO2 ratio. During nasal cannulae application, The FiO2 was calculated assuming an increase of FiO2 of 3% for every liter of O2 delivered. Oxygen flows with nasal cannulae did never exceeded 6 l/min. Venturi Mask FiO2 was calculated by available charts coupling O2 flow with the appropriate valve. Reservoir masks were set at 15 l/min and the relative FiO2 was considered as 90%. FiO2 during Helmet CPAP delivery was directly set before the inspiratory port of the circuit. FiO2 during NIV and IMV delivery was set directly on the ventilator.

14. Methods, in hospital treatment: Did the author pay attention to the phase of the disease before treatment with methylprednisolone?

Answer: We thank the Reviewer for raising the question. Criteria for systemic steroid administration were accurately judged for each patients by the attending physician. Patients satisfying the ATS/IDSA criteria for severe pneumonia and a hyper-inflammatory stage of the disease and no contra-indications for steroid treatment were administered a maximal dose of 1 mg/Kg. Specifically, criteria for initiation of methylprednisolone were: age <80 years, PaO2/FiO2 <250 mmHg; bilateral infiltrates, CRP >100 mg/L, and/or a diagnosis of acute respiratory distress syndrome (ARDS) according to the Berlin definition. This has now been detailed in the methods.

15. Page 9 Row 17: What kind of correction was made after performing this statistics?

Answer: We thank the Reviewer for raising the point. ANOVA and Kruskall-Wallis tests were corrected with Sidak adjustment to compare quantitative variables with normal and non-normal distribution, respectively. The latter has been added in the Statistics section.

16. Table 1: Is there no transitions between treatments ?

Answer: Data on respiratory support at admission refer to the conditions in which the initial ABG was performed and does not return the information about which patient experienced a worsening of respiratory conditions that required invasive or non invasive ventilatory support. The proportion of patients that needed CPAP or IMV and were intubated is also reported at the bottom of Table 1. We believe that reporting data also on respiratory support transitioning is outside the scope of the study and may a matter of confusion for the reader.

17. Page 17, Lines 54-56: It may be better not to provide results that highly correlates with indications. (for all other similar findings)

Answer: We thank the Reviewer for the comment. We agree that it appears pleonastic that treatments such as immunomodulatory and anti-inflammatory drugs were more often administered to patients with severe respiratory failure, although LMWH could not be the case. Indeed, the sentence indicated and also the following ones concerning steroids, in the present form, have some redundancy. Considering that they do not add to the meaning of the study, they were deleted.

18. Page 20, line 19: Did the author consider multicollinearity problem before the significant parameters were taken into account into multivariable regression analysis ?

Answer: We thank the Reviewer for the comment. No collinearity issue was found during the construction of the model.

19. Conclusions: Do we have enough data to say ' even in clinical stability'?

Answer: We agree with the Reviewer. Also according to Editors' suggestions (please see above), we toned down the conclusion section to go along the observational design of the study.

Reviewer 2

Dear Authors,

this study reported lower PaO2/FiO2 ratio as a predictor of in-hospital mortality in COVID-19 patients. This is a very interesting result, considering that risk stratification in Emergency settings is matter of concern for appropriate COVID-19 patients management. Another strength of the study is the prospective design. However several issues listed below should be addressed.

We thank the Reviewer for appreciating the study and its clinical implications

- Methods

1. How did you make the diagnosis of COVID-19? By using nasal-pharyngeal swabs? To exclude COVID-19 did you use single or double negative swabs?

Answer: We thank the Reviewer for having raised the point. COVID-19 diagnosis was obtained with a clinical presentation consistent with a SARS-CoV-2 infection and a positive nasal-pharyngeal swab. In case the first swab was negative, a second swab was repeated. In our case, due to local standard operating procedures, only patients with positive NP swabs were admitted to the participating units. The Methods section has been modified accordingly, also following the comments provided by Reviewer #1.

"The COVID-19 diagnosis was based on a positive nasopharyngeal swab collected in the emergency department. SARS-CoV-2 infection was proved by means of reverse transcriptase polymerase chain reaction (RT-PCR). In case a first swab was negative, and the clinical picture was highly suggestive for COVID-19, the swab was repeated."

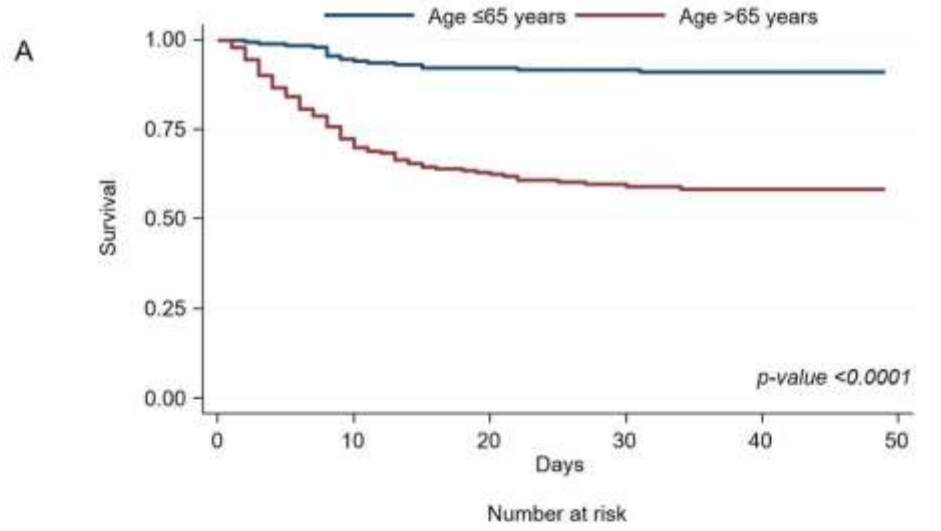
2. In addition, in which period were patients enrolled?

Answer: Patients were enrolled between March 7th and May the 7th 2020. Please see also the Methods section, 2nd line.

- Results

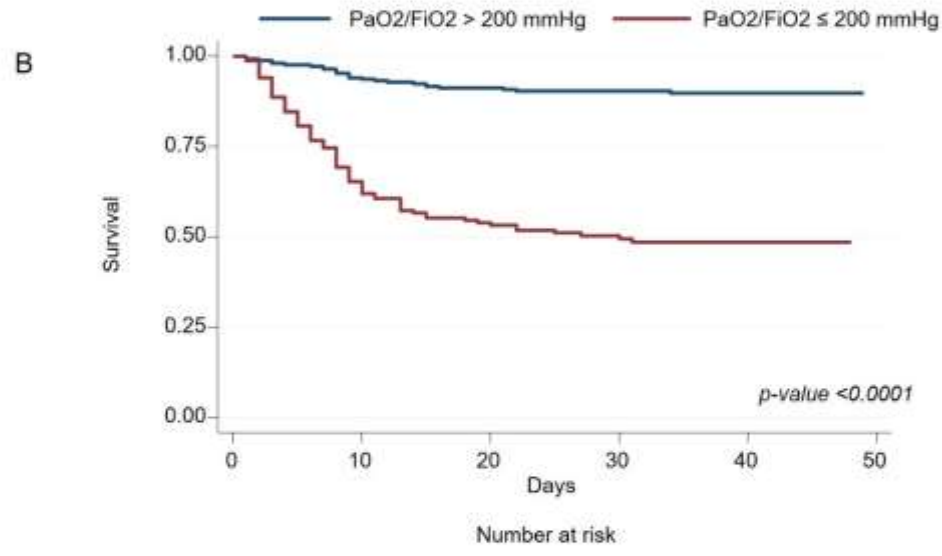
3. I suggest to perform Cox analysis by using categorical variables derived from continues variables (e.g Age > 65 years old) and add Kaplan-Meier for variables significantly associated with death. In addition you should run ROC curve to evaluate the performance of the model in predicting death.

Answer: We thank the Reviewer for the suggestions. We performed the Cox analysis transforming the categorical variable "Age" in > and ≤ 65 years old, and transforming the PaO2/FiO2 continuous variable in patients with a PaO2/FiO2 ratio ≤ and > 200 mmHg at admission. We also produced new Kaplan Meier graphs as new figures in replacement of previous figure 1 containing the three variable independently associated with mortality (Now Figure 1 panel A, B and C, please see below).



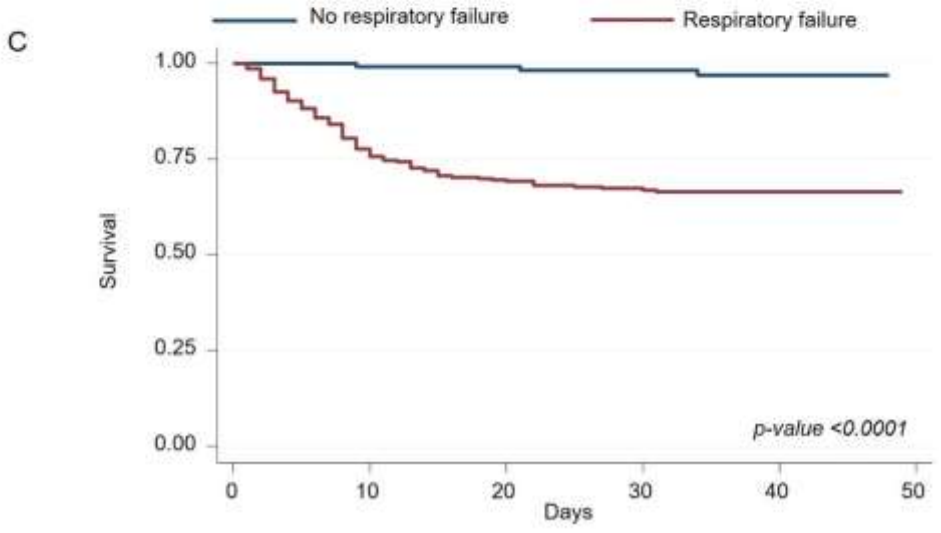
Number at risk

Age ≤65 years	205	194	183	157	61	0
Age >65 years	203	147	121	90	37	0



Number at risk

PaO2/FiO2 > 200 mmHg	252	237	220	186	78	0
PaO2/FiO2 ≤ 200 mmHg	150	98	78	56	16	0

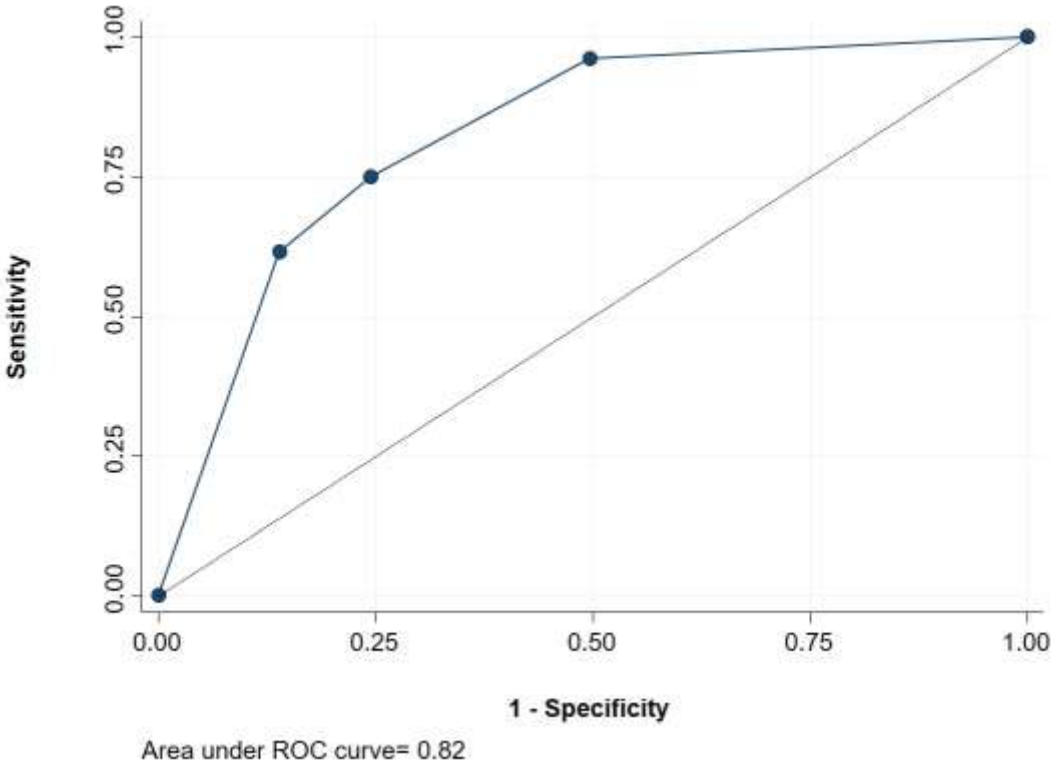


Number at risk

No respiratory failure	113	112	107	95	44	0
Respiratory failure	295	229	197	152	54	0

Based on the new statistical analysis and on the findings, we changed the Abstract, Results, Discussion and Conclusions sections accordingly

We here also report the ROC curve analysis for the new model. Given the limited clinical information that the ROC curve adds to the paper, and considering the amount of data already present in the manuscript, we preferred not to add the ROC curve in the Results.



VERSION 2 – REVIEW

REVIEWER	Celal Satici University of Health Science, Gaziosmanpasa Research and Training Hospital Istanbul/Turkey
REVIEW RETURNED	09-Sep-2020

GENERAL COMMENTS	Revised version of the manuscript looks better. Although the conclusion is not unexpected, it contributes to the literature as it is original.

REVIEWER	Davide Colombi Guglielmo da Saliceto" Hospital, Piacenza, Italy
REVIEW RETURNED	21-Sep-2020

GENERAL COMMENTS	Dear Authors, the article has improved after following almost all suggestions. I listed down below only few additional comments. Page 8, line 20: please amend "micoembolism".

	Page 11, line 43: you should add that you used Kaplan-Meier method and log-rank test to test the survival difference between group of patients. Page 12, line 12: did you define the acronym FEU previously in the text? Page 22, line 21: is the risk factor older age or age>65 years old as defined in abstract?

VERSION 2 – AUTHOR RESPONSE

Reviewer 1

Revised version of the manuscript looks better. Although the conclusion is not unexpected, it contributes to the literature as it is original.

Answer: We thank the Reviewer for appreciating the work done and for having contributed in ameliorating the manuscript.

Reviewer 2

Dear Authors,

the article has improved after following almost all suggestions. I listed down below only few additional comments.

Answer: We thank the Reviewer for his suggestions and for having contributed in improving the clinical meaning of the study.

Page 8, line 20: please amend "micoembolism".

Answer: Done

Page 11, line 43: you should add that you used Kaplan-Meier method and log-rank test to test the survival difference between group of patients.

Answer: We added the following sentence to the statistical methods:

“Kaplan-Meier survival curves were plotted to show differences for the outcome mortality, considering the confounding variables age, respiratory failure, and PaO2/FiO2; log-rank test was computed to assess the presence of any statistically significant differences.”

Page 12, line 12: did you define the acronym FEU previously in the text?

Answer: The abbreviation of fibrinogen-equivalent units (FEU) has been now added both in text at first use and in tables’ footnotes.

Page 22, line 21: is the risk factor older age or age>65 years old as defined in abstract?

Answer: it is correct. The risk factor was being older than 65 years old, as it is reported in the abstract and in table 4. The sentence has been changed as follows:

“[…] the multivariate analysis showed that the only independent risk factors were age >65 years (Hazard rate (HR) 3.41; 95% confidence interval (CI): 2.00-5.78, p-value <0.0001) […]”